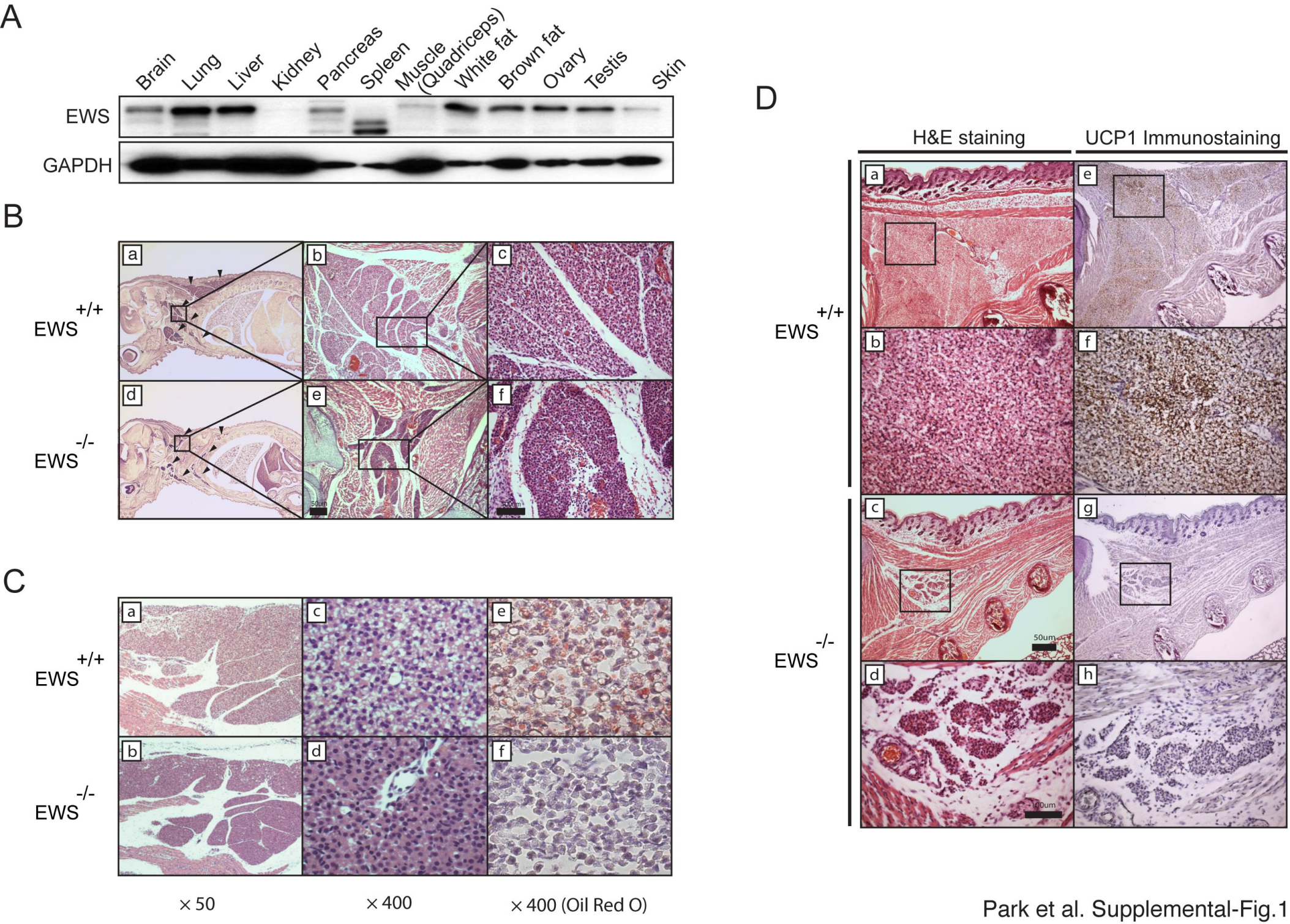
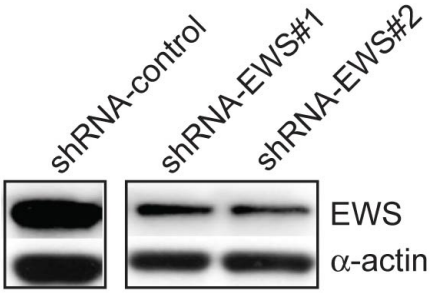
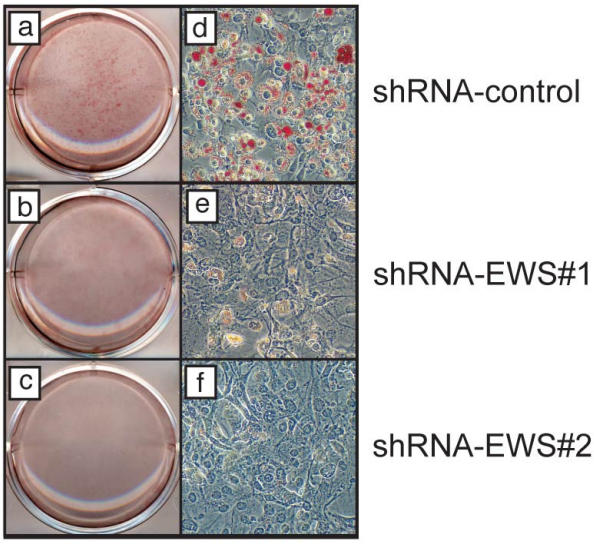


Park et al. Supplemental-Fig.1

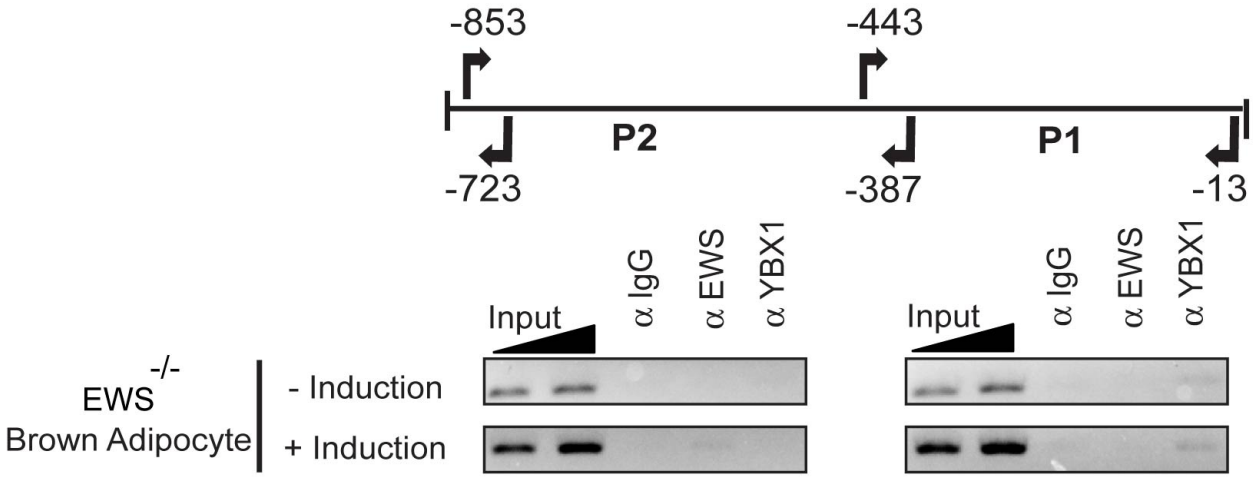
A


shRNA-control
shRNA-EWS#1
shRNA-EWS#2
EWS
α-actin

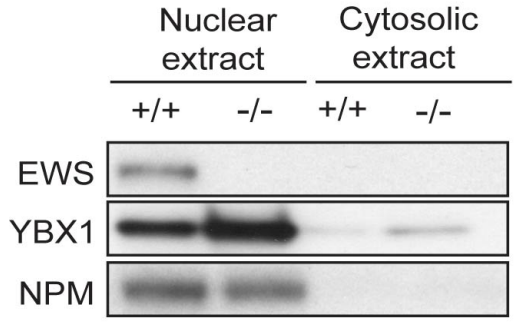
B


a
b
c
d
e
f
shRNA-control
shRNA-EWS#1
shRNA-EWS#2

A

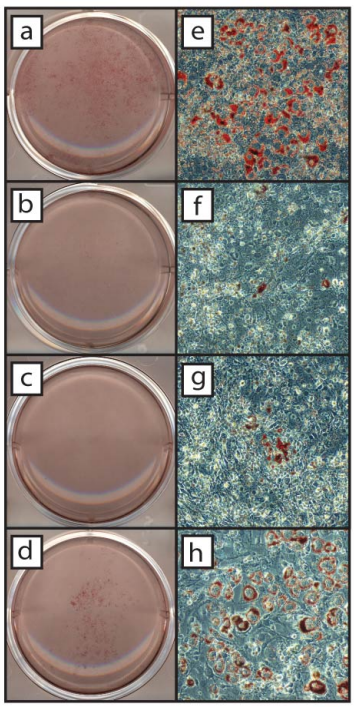


B



Park et al. Supplemental-Fig.3

A



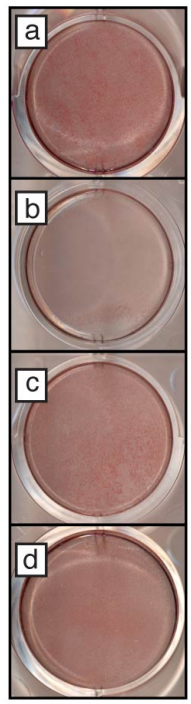
EWS$^{+/+}$ + GFP-control

EWS$^{-/-}$

EWS$^{-/-}$ + GFP-control

EWS$^{-/-}$ + EWS

B



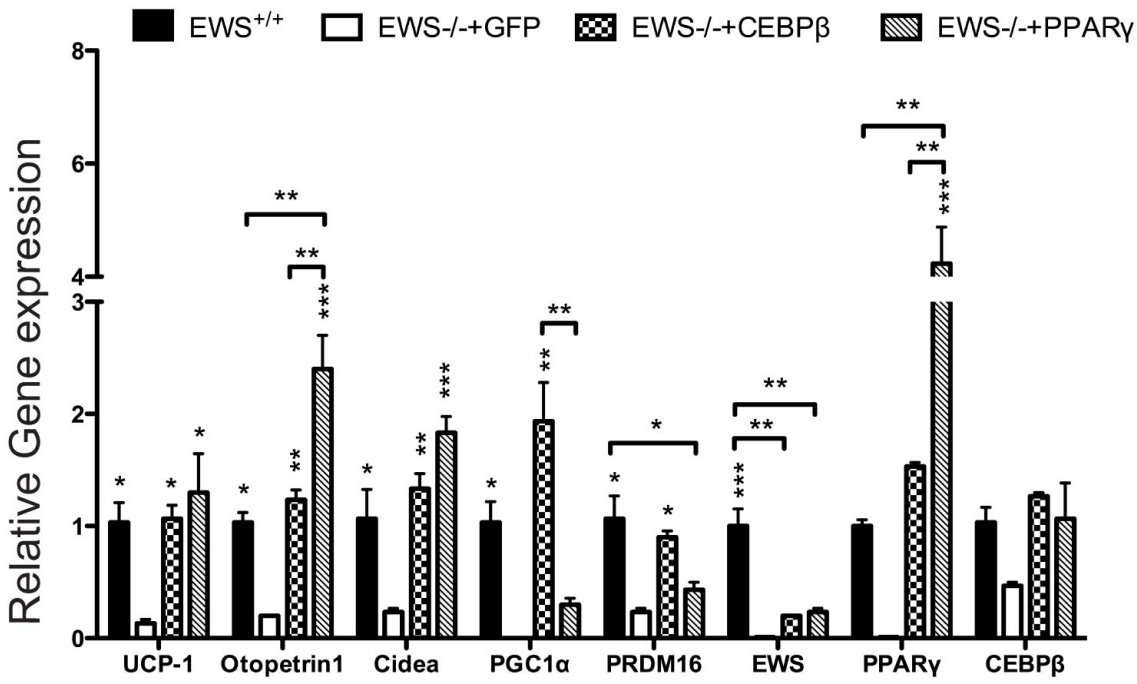
EWS$^{+/+}$

EWS$^{-/-}$ + GFP-control

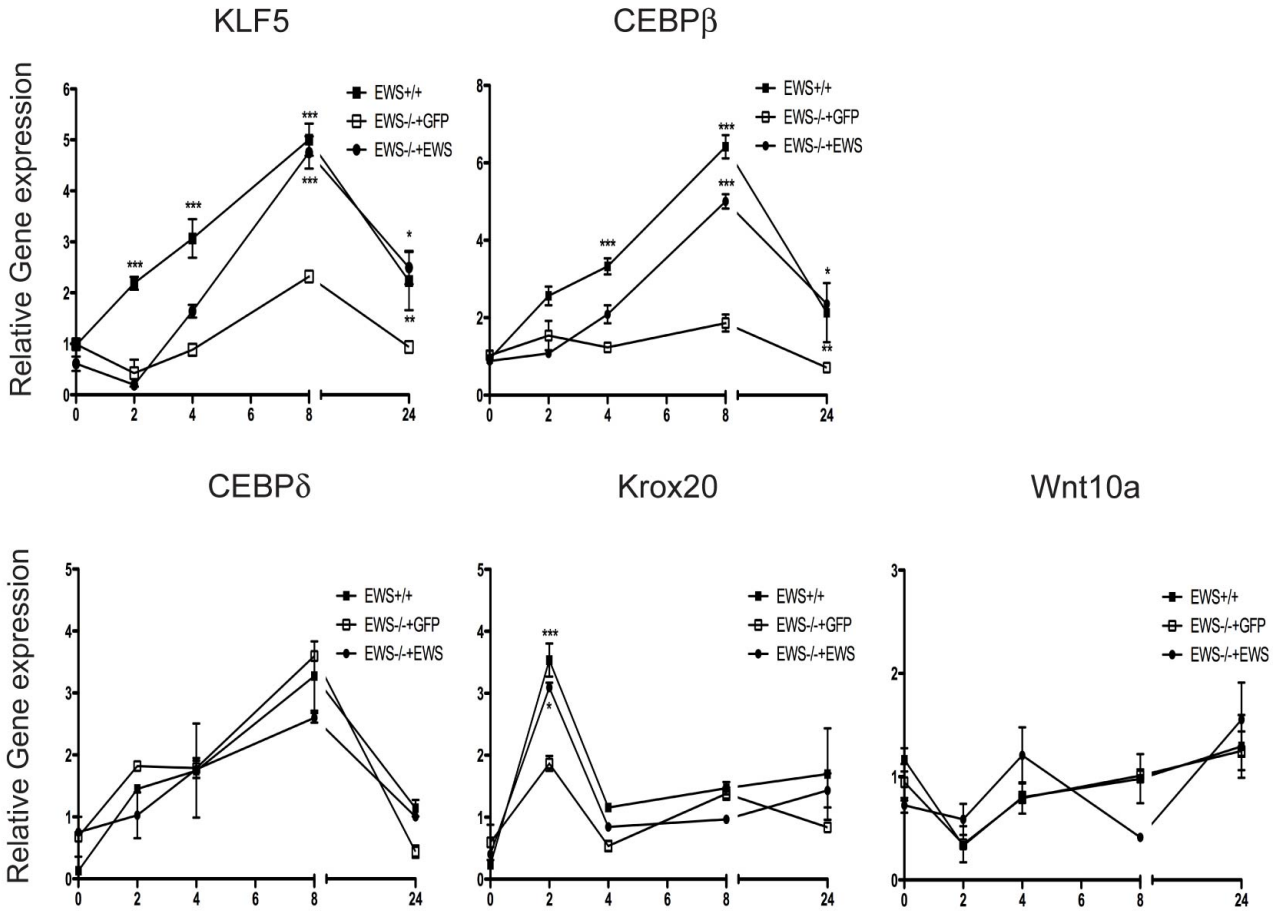
EWS$^{-/-}$ + CEBPβ

EWS$^{-/-}$ + PPARγ

C



A



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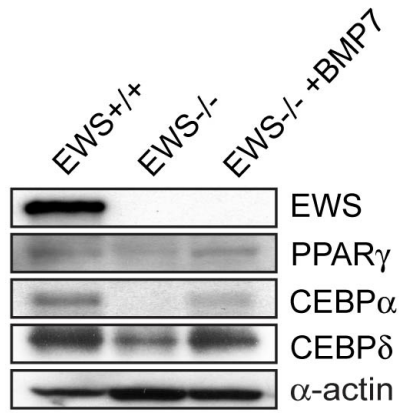
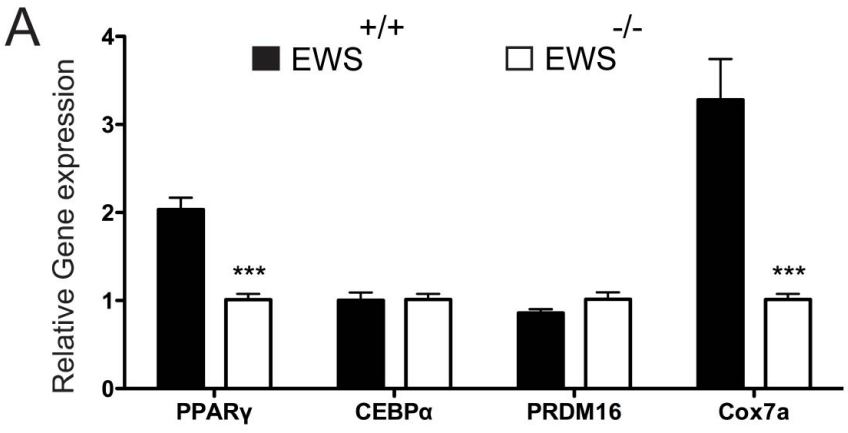
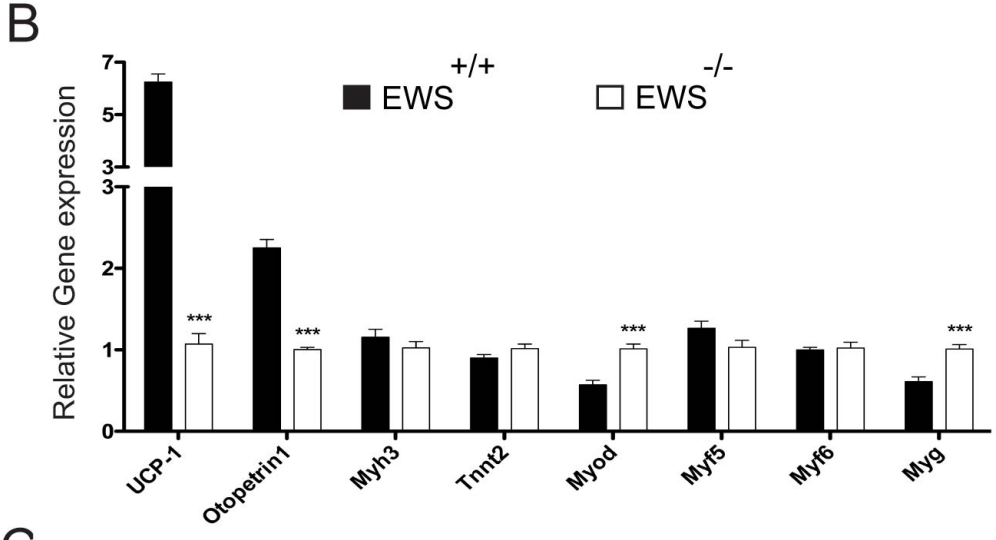
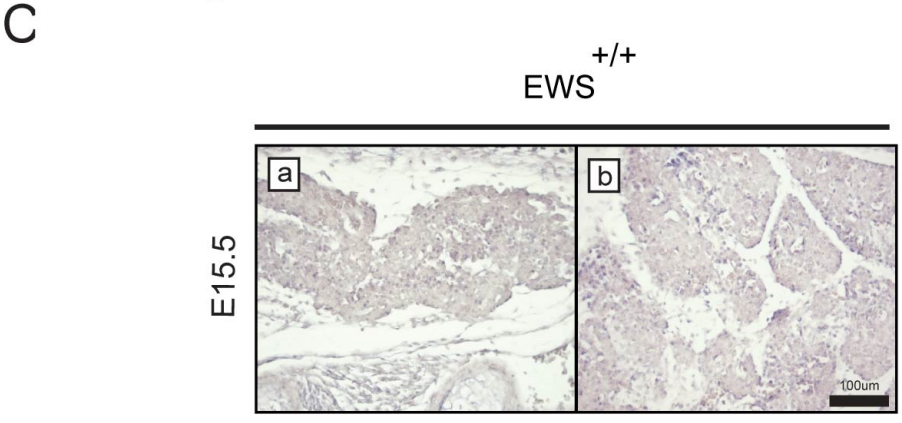
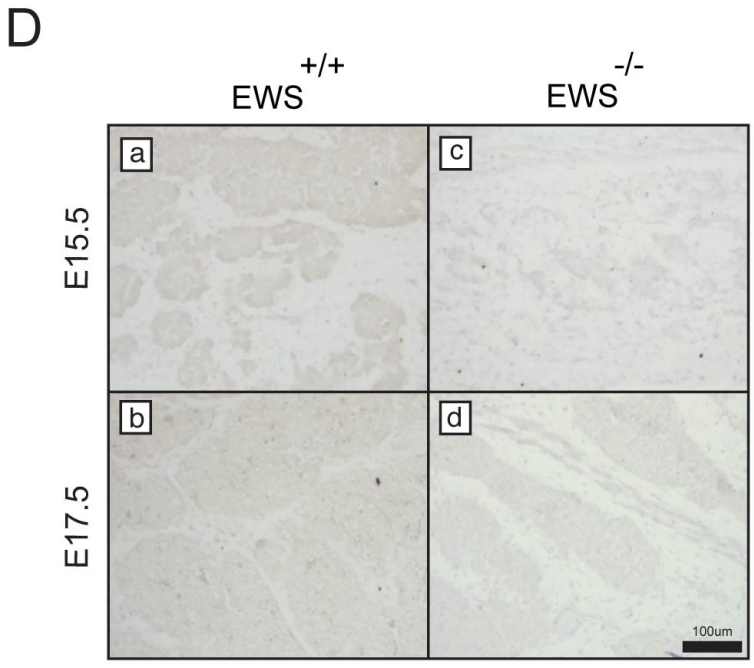


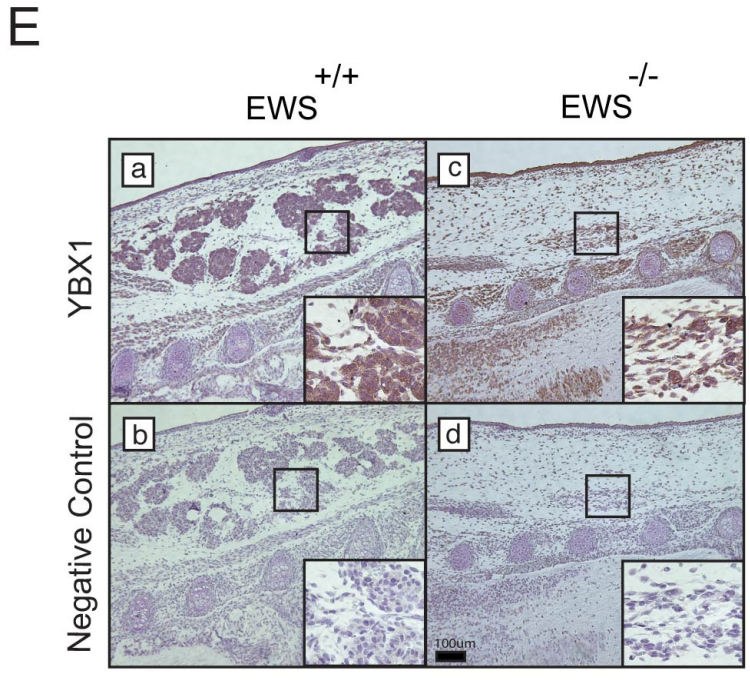
Park et al. Supplemental-Fig.5



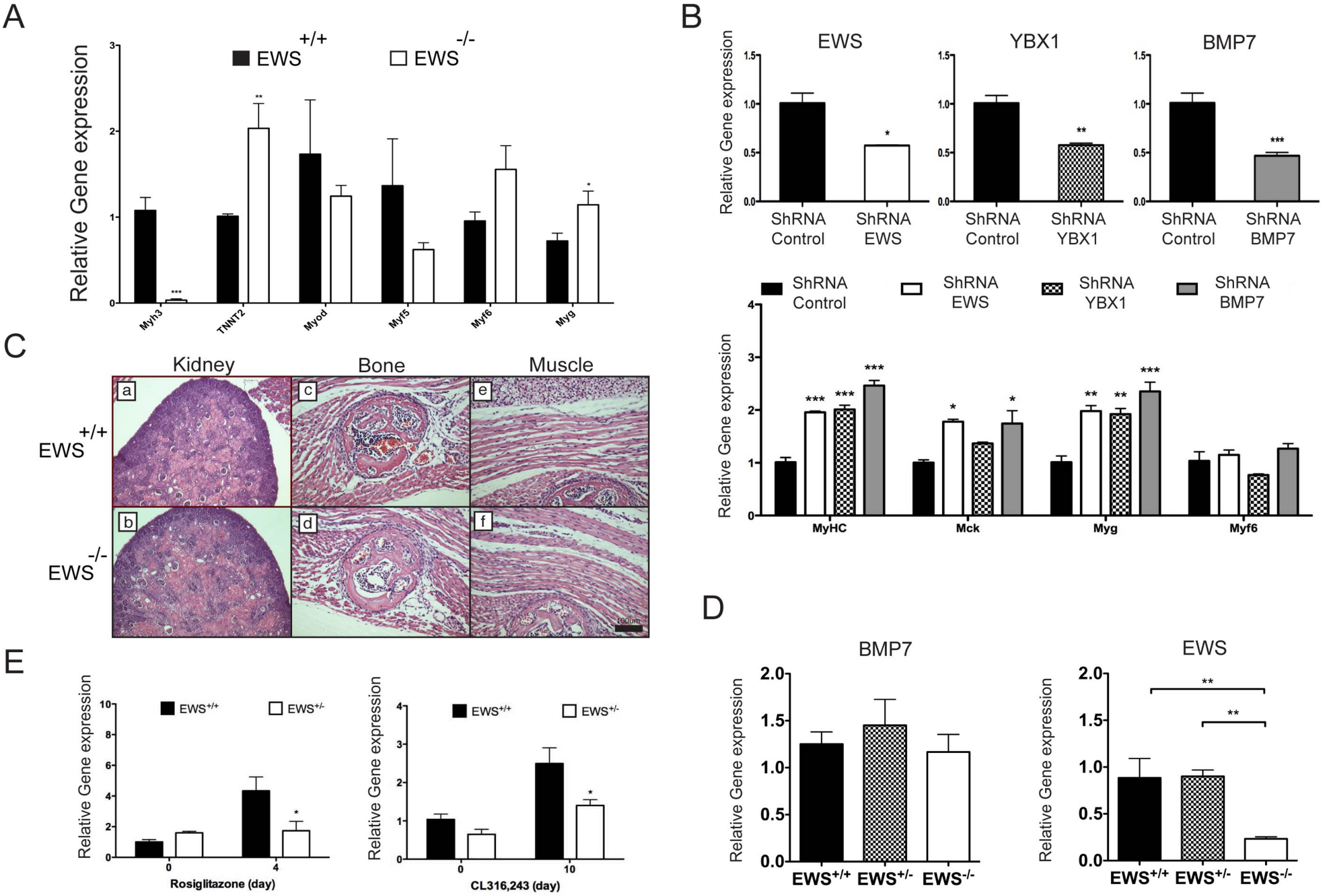








Park et al. Supplemental-Fig.6



Park et al. Supplemental-Fig.7

Supplementary Figure Legends

Supplementary Figure S1, Related to Figure 1. Genetic inactivation of *Ews* leads to arrest in BAT development. **A.** Expression of EWS in different tissues. Western blot analysis of EWS in 8-week old wildtype mouse tissues. **B.** H&E staining of paraffin-embedded sagittal sections of newborn pups (P0.5). Subscapular BAT (boxed area) is shown at higher magnification in the insets (C and F). Arrowheads indicate various BAT depots. Scale bars are indicated. **C.** Higher magnification images (C and D) of H&E stained BAT (P0.5) and representative images of cryosectioned BAT stained with Oil Red O (E and F). Magnifications are indicated. **D.** *Ews* inactivation leads to BAT defect in a mixed genetic background. *Ews*+/+ and *Ews*-/- in 129SvEv/Black Swiss mixed background newborns (P0.5) were fixed, paraffin-embedded and sagittally-sectioned. Representative images of H&E staining (A-D) and UCP1 immunohistochemistry (E-H) are shown. The boxed areas in A, C, E and G are shown in panels B, D, F and H, respectively. H&E analysis show smaller BAT size and lack of lipids in *Ews*-/- pups. Scale bars are indicated.

Supplementary Figure S2, Related to Figure 2. Depletion of *Ews* in brown preadipocytes leads to a block in adipogenesis. **A.** Wildtype immortalized brown preadipocytes were transduced with a lentivirus expressing shRNA against *Ews* (two independent, Sigma) or scrambled control (Sigma) and analyzed by Western blot analysis. **B.** Wildtype immortalized brown preadipocytes transduced with lentivirus containing shRNA against *Ews* (two independent) or scrambled control were cultured for 8 days in media containing adipogenic cocktail and stained with Oil Red O.

Supplementary Figure S3, Related to Figure 3. EWS is required for YBX1 recruitment to the mouse *Bmp7* promoter. **A.** ChIP analysis of the mouse *Bmp7* promoter region in *Ews*-/- brown preadipocytes with or without adipogenic cocktail (4hr). Note that YBX1 is only weakly recruited to the P1 region of mouse *Bmp7* promoter even after the adipogenic stimulation. **B.** YBX1 localization is not altered in *Ews* muant preadipocytes. Western blot anlaysis of nuclear and cytoplasmic extracts of *Ews*+/+ and *Ews*-/- preadipocytes. Antibodies are indicated. NPM (Nucleophosmin).

Supplementary Figure S4, Related to Figure 4. Complementation of *Ews, CEBPβ or PPARγ* in *Ews*-/- brown preadipocytes rescues brown adipogenesis and restores expression of critical adipogenic factors. **A.** *Ews*+/+ and *Ews*-/- preadipocytes transduced with a lentivirus expressing either *Ews* or *GFP* (control) were cultured for 8 days in media containing adipogenic cocktail and stained with Oil Red O. **B-C.** *Ews*+/+ and *Ews*-/- preadipocytes transduced with a lentivirus expressing *CEBPβ, PPARγ* or *GFP* (control) were cultured for 8 days in media containing adipogenic cocktail and stained with Oil Red O **(B)** or analyzed by qRT-PCR for brown adipocyte markers (**C**). Three independent experiments were performed and data are represented as means ±s.e.m. *p<0.05, **p<0.01, ***p<0.001.

Supplementary Figure S5, Related to Figure 4. A. *Ews*+/+ or *Ews*-/- preadipocytes transduced with a lentivirus expressing either *Ews* or *GFP* (control) were stimulated with adipogenic cocktail for indicated hours (0 to 24 hr) and indicated transcripts were analyzed by qRT-PCR. Data are represented as means ±s.e.m. One-way ANOVA, *p<0.05, **p<0.01, ***p<0.001. **B.** *Ews*+/+ or *Ews*-/- brown preadipocytes were cultured in media containing adipogenic cocktail

with or without recombinant BMP7 (100 ng/ml) for 2 days, and continuously cultured in adipogenic cocktail-only containing media for 6 more days. Subsequently, cells were harvested and analyzed by Western Blot analysis.

Supplementary Figure S6, Related to Figure 5. Reduced expression of BAT markers in E18.5 *Ews*-null BAT. Interscapular BATs from E18.5 *Ews*+/+ and *Ews*-/- littermate embryos (n=3 per genotype) were harvested and analyzed by qRT-PCR for BAT **(A)** as well as muscle markers **(B)**. Data are represented as means ±s.e.m. *p<0.05, **p<0.01, ***p<0.001. **C-D.** *Bmp7* RNA *in-situ* analysis of embryonic BAT. RNA *in-situ* analysis was performed on E15.5 or E17.5 *Ews*+/+ and *Ews*-/- embryos with antisense **(C)** or sense **(D)** mouse *Bmp7* RNA probes. BATs from two independent wildtype E15.5 embryos are shown in **(C)**. **E.** YBX1 is expressed in E15.5 brown fat precursor cells. Paraffin-embedded sections of E15.5 Ews+/+ and Ews-/- embryos were immunostained with anti-YBX1 or mouse IgG (negative control), followed by incubation with secondary antibody coupled to HRP. Insets show 40X magnification of the boxed areas. Scale bar is indicated.

Supplementary Figure S7, Related to Figures 6 and 7. Ectopic expression of myogenic genes in *Ews*-null brown preadipocytes. **A.** Elevated expression of muscle markers in *Ews*-null brown preadipocytes. *Ews*+/+ or *Ews*-/- preadipocytes were cultured for 8 days in media containing adipogenic cocktail and total RNA was analyzed by qRT-PCR. Note increased expression of muscle (*Tnnt2* and *Myg*) genes in *Ews*-/- preadipocytes. Three independent experiments were performed and data are represented as means ±s.e.m. *p<0.05, **p<0.01, ***p<0.001. **B.** Wildtype brown preadipocytes were transduced with lentivirus expressing shRNA against GFP

(control), BMP7, EWS or YBX1. Following stimulation with myogenic differentiation media (2% horse serum) for 6 days, total RNA was isolated and expression of myogenic genes was analyzed by qRT-PCR. Data were normalized against *β-Actin* and relative expression of each transcript was calculated by comparative Ct method. One-way ANOVA, *p<0.05, **p<0.01, ***p<0.001. **C.** H&E analysis of kidney, bone and muscle from *Ews*+/+ and *Ews*-/- newborns (P0.5). Scale bar is indicated. **D.** E18.5 kidneys from littermate embryos (n=3 embryos/genotype) were harvested and total RNA was isolated. Tails were used for retrospective genotyping. Relative quantity of *Bmp7* and *Ews* transcripts was analyzed by qRT-PCR and normalized against *β-Actin*. One-way ANOVA, **p<0.01. **E.** qRT-PCR analysis of *Tmem26* in inguinal fat fads from *Ews*+/+ or *Ews*+/- mice following either four daily i.p. injections with PBS or rosiglitazone (10mg/kg) (n=3) or ten daily i.p. injections with PBS or CL316,243 (1mg/kg) (n=3). Data are represented as means ±s.e.m. Two-way ANOVA, *p<0.05.

Supplementary Experimental Procedures

siRNA

The following siRNAs were purchased:

mEws-1: 5'-GCA GUU ACU CUC AGC AGA AdTdT-3' (Sigma)

mEws-2: 5'-GAG ACU AGU CAA CCU CAA UdTdT-3' (Sigma)

mEws-3: 5'-CUG ACA ACA GUG CAA UUU AdTdT-3' (Sigma)

mYbx1-1: 5'-AGA AGG UCA UCG CAA CGA AdTdT-3' (Ambion)

mYbx1-2: 5'-CCA CGC AAU UAC CAG CAA AdTdT-3' (Ambion)

Scrambled Silencer Negative Control#1 (Ambion)

Genotype Primers

Multiplex PCR was performed on tail biopsies with the following primers:

Ews Forward: 5'-TGG ATC CTA CAG CCA GGC TCC-3'

Ews Reverse-wt: 5'-TGC TCG CTA GTG CTC TGT GAG C-3'

Ews Reverse-mut: 5'-TGG CGG ACT AAT TCA TCT GAC C-3'

ChIP primers

P1: Bmp7(-443), 5'-GGT GGG CAC TCG GTA AAT A-3'; Bmp7(-13) 5'-AGC ACA GCA ACT CCA GAG AG-3'

P2: Bmp7(-853), 5'-TGG GGG AGT GAA GTG TAG AA-3'; Bmp7(-387) 5'-CCA ATG GCT TCA TTC ATT CCT-3'

P3: Bmp7(-723), 5'-TGC TAT CCT GGC TTT TGT TC-3'**;** Bmp7(-1144) 5'-GGT GGG GTT GTT AGA CAG TG-3'

P4: Bmp7(-1101), 5'-CCT GAC ATG AGT CCT TTG GT-3'; Bmp7(-1570) 5'-AAG GAG CCC TGA GAG ACC TA-3'

Real-time qRT-PCR primers and TaqMan probes

All TaqMan probes and primers were purchased from Applied Biosystems. EWS (Mm00783962_sH), CEBPα (Mm01265914_s1), CEBPβ (Mm00843434_s1), CEBPδ (Mm00786711_s1), PPARγ (Mm01184322_m1), UCP1 (Mm00494069_m1), PGC1α (Mm01208835_m1) and GAPDH (Mm03302249_g1).

The following primers were purchased (MWG Operon) for SYBR Green PCR:

Prdm16 -Forward	5'-CAGCACGGTGAAGCCATTC-3'
Prdm16 -Reverse	5'-GCGTGCATCCGCTTGTG-3'
Elovl3/Cig30 -Forward	5'-TCCGCGTTCTCATGTAGGTCT-3'
Elovl3/Cig30 -Reverse	5'-GGACCTGATGCAAACCCTATGA-3'
Cidea -Forward	5'-TGCTCTTCTGTATCGCCCAGT-3'
Cidea -Reverse	5'-GCCGTGTTAAGGAATCTGCTG-3'
Lkb1 -Forward	5'-CTACTCCGAGGGATGTTGGA-3'
Lkb1 -Reverse	5'-GAT AGGTACGAGCGCCTCAG-3'
Klf5 -Forward	5'-CATGCCAAGTCAGTTTCTTCCA-3'
Klf5 -Reverse	5'-TTCCAGGCTCTGAGCTTGGT-3'
Cebpβ -Forward	5'-AAGCTGAGCGACGAGTACAAGA-3'
Cebpβ -Reverse	5'-GTCAGCTCCAGCACCTTGTG-3'
Cebpδ- -Forward	5'-CGACTTCAGCGCCTACATTGA-3'
Cebpδ -Reverse	5'-CTAGCGACAGACCCCACAC-3'
Krox20 -Forward	5'-TGACTATTGTGGCCGCAAGTT-3'
Krox20 -Reverse	5'-TTCTGCCGAAGGTGGATCTT-3'
Wnt10a -Forward	5'-TCCAAGAAATCCCGAGAGAA -3'
Wnt10a -Reverse	5'-CACTTACGCCGCATGTTCT-3'
Otopetrin1 -Forward	5'-ACTAGGACCCCGTCGAATCT-3'
Otopetrin1 -Reverse	5'-ACCATGCTCTACGTGCTGTG-3'
Mck -Forward	5'-GCAAGCACCCCAAGTTTGA
Mck -Reverse	5'-ACCTGTGCCGCGCTTCT-3'
MyHC -Forward	5'-TCCAAACCGTCTCTGCACTGTT-3'
MyHC -Reverse	5'-AGCGTACAAAGTGTGGGTGTGT-3'

Myh3 -Forward	5’-CTTCACCTCTAGCCGGATGGT-3’
Myh3 -Reverse	5’-AATTGTCAGGAGCCACGAAAAT-3’
Tnnt2 -Forward	5’-GCGGAAGAGTGGGAAGAGACA-3’
Tnnt2 -Reverse	5’-CCACAGCTCCTTGGCCTTCT-3’
MyoD -Forward	5’-CGCCACTCCGGGACATAG-3’
MyoD -Reverse	5’-GAAGTCGTCTGCTGTCTCAAAGG-3’
Myf5 -Forward	5’-CAGCCCCACCTCCAACTG-3’
Myf5 -Reverse	5’-GGGACCAGACAGGGCTGTTA-3’
Myf6 -Forward	5’-ATCAGCTACATTGAGCGTCTACA-3’
Myf6 -Reverse	5’-CCTGGAATGATCCGAAACACTTG-3’
Myog -Forward	5’-AGCGCAGGCTCAAGAAAGTGAATG-3’
Myog -Reverse	5’-CTGTAGGCGCTCAATGTACTGGAT-3’
Cox7a -Forward	5’-CTGAGGACGCAAAATGAGG-3’
Cox7a -Reverse	5’-TGGCTTCTGGTAGATGAGCTAAA
β-actin -Forward	5’-TTGCTGACAGGATGCAGAAG-3’
β-actin -Reverse	5’-GAAAGGGTGTAAAACGCAGC-3’